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Assessment of pesticides cytoxicity by means of bioelectric profiling of mammalian cells

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ABSTRACT

In the present study an innovative tool was performed for assessing pesticide cytoxicity of different pesticide groups by using the bioelectric response patterns of neuroblastoma and fibroblast cell lines. Two different modes of monitoring the bioelectric response, potentiometry and amperometry, were applied in cell suspensions exposed to the pesticides. A unique pattern for each cell type was observed depending on the mode of measurement and the pesticide group. The results with amperometry were more reproducible than potentiometry. The general results of the study demonstrate the possibility of using bioelectric profiling as an approach for developing novel cytoxicity assays with the advantage of high speed and the ability to respond to an extended range of toxicants.

1. Introduction

Mammalian cells are a unique tool for in vitro toxicity assays, since they represent, with a fairly high degree of accuracy, the actual biological targets of many environmental pollutants or other compounds with proven or suspected toxicity. In a broader sense, higher eukaryotic cells have been recruited as biorecognition elements either in advanced cell-based biosensors (CBBs) or in cell-based assays (CBAs) (Banerjee et al., 2013). Whichever the case, the final output of any cell-based test system is the measurement of the deviation of a predetermined cellular function from control, “baseline” levels as a result of exposure to a toxic compound. This information can be used either for assessing the biotoxic properties of the assayed sample or, in the case of CBBs, for determining the analyte in question, mainly qualitatively and, preferably, quantitatively as well.

The choice of the measured cellular function is critical to the successful development of a practically useful CBA. The following criteria should be considered when evaluating different cellular parameters for building appropriate working assay principles:

(i) A sufficiently high speed of measurement, low cost and ease-of-use, to satisfy high throughput requirements: most currently available methods for measuring toxic effects on mammalian and other cell types are associated with either optical (dye-based) (Sérandour et al., 2012) or electric (impedance spectrometry) (Wegener et al., 2000) working principles implemented in multi-step processes, which demand several hours before enough data are collected. The availability of skilled personal for the operation of such systems is an additional drawback.

(ii) Ability to respond to a broad spectrum of toxicants and providing, at the same time, a more or less unique pattern of response against individual compounds (or at least groups), so that selective detection is feasible. So far, this has been achieved only against a very limited number of organic toxicants and pathogens, thanks to the advent of technologies such as CANARY (Rider et al., 2003) and the Molecular Recognition through Membrane Engineering (Moschopoulou et al., 2012). Still, the vast number of pollutants of interest has prohibited the rapid development of cell lines with selective biorecognition elements.

(iii) Finally, the ability to provide quantitative or semi-quantitative data rather than just qualitative information from screening an unknown sample.

Bioelectric assay methods based on mammalian cells have gained considerably in popularity over the past few years. Model approaches are represented by the measurement of (1) extracellular recordings from cardiac myocytes cultured on microelectrode arrays (MEA) (Natarajan et al., 2006), (2) the impedance of adherent cells in culture (Curtis et al., 2009; Schwarzenberger et al., 2011) and (3) membrane potential of cells immobilized in a gel matrix (the Bioelectric Recognition Assay – BERA) (Kintzios et al., 2001a; Mavrikou et al., 2008).

In the present report we describe a new approach for the simple, rapid and cost-efficient measurement of bioelectric patterns of different mammalian cell lines in response to toxicants belonging to different chemical groups. Our approach is based on a modification of the Bioelectric Recognition Assay, with the following novelties: (a) cells

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used as biorecognition elements are suspended, not gel immobilized, thus drastically reducing the cost of each assay (b) two different aspects of the cellular bioelectric response are measured, namely the potential and the combined resistance + capacitance of the cell suspension. In this way, the amount of information derived from each assay is considerably increased. We demonstrate that the proposed approach can be applied to the high throughput, differential characterization of the toxic effect of selected pesticide groups on mammalian cells.

2. Materials and methods

2.1. Materials

The three categories of pesticides that we used are carbamates, organophosphates and pyrethroids. The mechanism of action of carbamates and organophosphates is the inactivation of the enzyme acetylcholinesterase (Fukuto, 1990). On the other hand, pyrethroids can affect the permeabilities to sodium and potassium ions (Narahashi, 1971). Both the pesticide groups and the individual compounds which were used in the present study were selected on the basis of the occurrence of the residues of the respective pesticides as well as their commercial availability. For this purpose, an excessive survey was conducted in four basic axes, i.e. market research, literature, official authorities and official reports. Based on the results of the survey, a specific formulation was created for each pesticide group, as presented in Table 1. In this way, each group contained pesticidal compounds which are representative (i) of different levels of solubility in water or polar solvents, since nonpolar solvents are not suitable for use with cellular biorecognition elements and (ii) of actual compounds currently used in European agriculture. The use of pesticide mixtures, each corresponding to an individual group, allows for safeguarding a group-specific cell response irrespective of minor differences in the mode of action among pesticides belonging to the same group. In addition, in real-life agricultural applications commonly mixtures of 2–5 pesticides are sprayed instead of single compounds. Therefore, the use of mixtures was deemed more appropriate for our experimental approach. Due to the fact that individual pesticides are associated with different Minimum Residue Level (MRL) values, we decided to create the three different mixtures (corresponding to the three different pesticide groups) by adding pesticides at concentrations ranging from the lowest to the highest MRL values commonly associated with residue analysis (0,0025–0,05 ppm). In this way, the present cell-based assay was developed on the principle of cell exposure to increasing cumulative pesticide accumulation within each group from a minimum to levels exceeding the MRL, in reflection of the actual field conditions regarding residue distribution in real samples.

Table 1
Composition of target pesticide mixtures used in the cell bioelectric profiling experiments.

Group	Target compounds
Organophosphates	Acephate azinphos methyl chlorpyriphos methyl dimethoate malathion mathamidophos pirimiphos methyl profenofos triazophos
Carbamates (+bezimidazolecarbamates)	Carbendazim carbofuran methomyl oxamyl iprodione
Pyrethroids	Acrinathrin cyfluthrin cyhalothrinlamda cypermethrin deltamethrin fenpropathrin fenvalerate flucythrinate

Commercial formulations were used for preparing standard pesticide solutions daily in double distilled water. All other reagents were purchased from Fluka (Switzerland). Cells were cultured in Dulbecco's medium with 10% fetal bovine serum (FBS), 1 U μg^{-1} antibiotics (penicillin/streptomycin) and 2 mM L-glutamine. Cells were detached from the culture and concentrated by centrifugation (2 min, 1200 rpm, 25 °C), at a density of 2.5×10^6 mL^{-1}. During each assay (see below, 2.3) cells were used at a density of 1000 μL^{-1}.

The following cell cultures were used in the present study: (a) Two immortalized mammalian fibroblast cell lines: African green monkey kidney (Vero) and Hamster adult kidney (HaK), (b) Two neuroblastoma cell lines: Mouse neuroblastoma (N2a) and Human neuroblastoma (SK-N-SH).Cell cultures were originally provided from LGC Promochem (UK). In this way, a relatively wide representation of mammalian cell targets was used for assessing the effect of exposure to pesticides.

The two neuroblastoma cell lines (N2a, SK-N-SH), being neuronal, are natural targets of all three pesticide groups, due to the inhibition of either acetylcholine esterase (AChE) (organophosphates, carbamates) or ion channels (pyrethroids). Under control conditions (no pesticides present), when acetylcholine is added to the cells, it causes a temporary depolarization of the cell membrane (excitation), which is rapidly cancelled out by the specific cellular mechanisms. However, when pesticides are present, they inhibit these mechanisms (such as AChE), thus allowing for a continuous stimulation of the neural cells. The ensuing membrane depolarization can be measured by appropriate methods (Mavrikou et al., 2008; Voumvouraki and Kintzios, 2011).

On the other hand, non-neuronal fibroblast cell lines (Vero, HaK) are also able to respond to pesticides with a considerable change of the cell membrane potential, as previously shown for Vero cells treated with either organophosphates or carbamates (Flampouri et al., 2010), an effect that has been partially attributed to pesticide interactions with the zinc receptors on the kidney cells (Houtani et al., 2005).

2.2. Biosensor device

Both potentiometric and amperometric measurements were received by means of a customized, 8x channel potentiostat (Uniscan, Buxton, UK). The system allowed for measuringelectric signals from cells suspended on the screen-printed working electrode and allowing for high throughput screening and high speed of assay (*duration: 3* min). The system is presented as a lab based bench top based on a modular potentiostat design. A connection interface including a replaceable guide allowed inserting electrode strips directly into the instrument, utilizing one electrode strip per channel. The sensor strips plug directly into the front panel of the instrument channels via a bespoke sensor connector (Fig. 1). Each electrode strip comprised a 0.5 mm thick ceramic substrate with three screen printed electrodes (working electrode – WE, reference electrode – RE and counter electrode – CE). In order to facilitate high throughput screening, DRP-8 × 110 disposable sensor strips (WE: carbon, RE: Ag/AgCl) bearing eight electrode pairs (corresponding to eight measurement channels) were purchased from DropSens (Asturias, Spain).

2.3. Bioelectric profiling assay process

The following procedure was used for recording cellular bioelectric responses against pesticides: cultured cells in suspension were added first on the top of each of the eight carbon screen-printed electrodes